Origin of life: LUCA and extracellular membrane vesicles (EMVs)

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Abstract: Cells from the three domains of life produce extracellular membrane vesicles (EMVs), suggesting that EMV production is an important aspect of cellular physiology. EMVs have been implicated in many aspects of cellular life in all domains, including stress response, toxicity against competing strains, pathogenicity, detoxification and resistance against viral attack. These EMVs represent an important mode of inter-cellular communication by serving as vehicles for transfer of DNA, RNA, proteins and lipids between cells. Here, we review recent progress in the understanding of EMV biology and their various roles. We focus on the role of membrane vesicles in early cellular evolution and how they would have helped shape the nature of the last universal common ancestor. A membrane-protected micro-environment would have been a key to the survival of spontaneous molecular systems and efficient metabolic reactions. Interestingly, the morphology of EMVs is strongly reminiscent of the morphology of some virions. It is thus tempting to make a link between the origin of the first protocell via the formation of vesicles and the origin of viruses.

Extracellular membrane vesicles (EMVs) in the three domains

The secretion of EMVs from cell surfaces is a universally conserved process that occurs in eukaryotes, bacteria and archaea (Raposo & Stoorvogel 2013). Although initially dismissed as 'cellular dust' extracellular vesicles have emerged as important mediators of inter-cellular communication between cells from all three domains.

These spherical structures are known to contain a variety of molecules including proteins, lipids and nucleic acids. Their diverse biological functions have been extensively documented (see Table 1). The list of functions attributed to EMVs includes binding and delivery of nucleic acids, transport of virulence factors, ridding the cell of toxic envelope proteins and tumorigenesis (Deich & Hoyer 1982; Dorward *et al.* 1989; Kadurugamuwa & Beveridge 1997; Kesty *et al.* 2004; Renelli *et al.* 2004; McBroom & Kuehn 2007; Valadi *et al.* 2007; Soler *et al.* 2008; Aldick *et al.* 2009; Ellis & Kuehn 2010; Camussi *et al.* 2011; Rak & Guha 2012; Gaudin *et al.* 2013).

The origin, nature and features of EMVs are diverse, and at present there is no consensus on the nomenclature of cell-derived vesicles, with many names being used interchangeably in the literature (Gould & Raposo 2013). This is largely due to an incomplete understanding of extracellular vesicle biogenesis, inconsistencies in extracellular vesicle purification protocols and a lack of detailed vesicle characterization.

EMVs in eukaryotes

EMVs in eukaryotes have been classified from anywhere between 2 and 6 groups based either on their cellular origin, biological function or method of formation (Cocucci *et al.* 2009; Théry *et al.* 2009; Beyer & Pisetsky 2010; Mathivanan *et al.* 2010). Based on their mode of biogenesis, eukaryotic EMVs can be classified into three main groups: microvesicles (50–1000 nm), exosomes (40–100 nm) and apoptotic bodies (500–2000 nm) (El Andaloussi *et al.* 2013). Here we focus on microvesicles and exosomes as these two groups are common to all classifications. Microvesicles are formed by budding off from the plasma membrane whereas exosomes are derived from the endolysosomal pathway. The cargoes of both EMV types include cytoplasmic proteins, lipid raft–interacting proteins and RNAs (mRNA, microRNA and other non-coding RNAs) (El Andaloussi *et al.* 2013). Through transfer of their molecular contents, EMVs are capable of altering the function of recipient cells.

EMVs have been identified in body fluids such as serum, saliva, amniotic fluid, synovial fluid, breast milk and urine (Pisitkun *et al.* 2004; Keller *et al.* 2007; Lakkaraju & Rodriguez-Boulan 2008; Kosaka *et al.* 2010; Michael *et al.* 2010). Increasing evidence suggests that both exosomes and microvesicles play a fundamental biological role in the regulation of normal physiological processes (Raposo *et al.* 1996; Del Conde *et al.* 2005; Ratajczak *et al.* 2006; Gatti *et al.* 2011). The

Table 1. *Overview of the main characteristics of different types of EMVs from the three domains of life (table adapted from van der Pol et al. 2012; El Andalousi et al. 2013)*

	Vesicle type	Origin	Size	Contents
Eukaryotes	Exosomes	Endolysosomal pathway	40–120 nm	mRNA, microRNA (miRNA); cytoplasmic and membrane proteins including receptors and major histocompatibility complex (MHC) molecules
	Micro vesicles	Cell surface; outward budding of cell membrane	50–1000 nm	mRNA, miRNA, non-coding RNAs, cytoplasmic proteins and membrane proteins.
	Apoptosis	Cell surface of all cell types	500–2000 nm	Nuclear fractions, cell organelles, histones and DNA.
Bacteria	Extra cellular vesicles	Cytoplasmic membrane	20–250 nm	Cytosolic proteins
	Outer membrane vesicles	Outer membrane	20–300 nm	Lipo polysaccharide; phospholipids; nucleic acids, cytosolic or inner membrane proteins; OmpA in *E. coli*.
Archaea	Membrane vesicles	Cytoplasmic membrane	50–230 nm	In Sulfolobus: endosomal sorting complex required for transport (ESCRT)I, ESCRT-II, Vps4, vWA, thiosulfate sulphur transferase, flotillin, disulphide oxidoreductase, S-layer proteins and archaeal tetraether lipids. In Thermococcales: nucleic acids and proteins.

release of EMVs, is also enhanced in disease processes such as cancer (Inal *et al.* 2013). The novel discoveries in the field of EMVs, especially in multicellular eukaryotes have led to the creation of an international society for the study of extracellular vesicles and the creation of two new journals.

EMVs in bacteria

For many decades the formation of EMVs in prokaryotes was solely focused on bacteria (Kuehn & Kesty 2005; Ellis & Kuehn 2010; Kulp & Kuehn 2010). In bacteria with double membranes (referred to as diderm bacteria) they are generally derived from the outer membrane (OM) which is distinct from the cytoplasmic membrane, and are referred to as OMVs (Kulp & Kuehn 2010). During the growth the OM 'blebs' outwards and pinches off, forming vesicles (20–250 nm) that are spherical portions of OM with lumenal periplasmic content. Thus, the composition of OMVs reflects components of the OM and periplasm, for example, soluble proteins, integral membrane proteins, lipoproteins and glycolipids (Beveridge 1999; Horstman & Kuehn 2000; Schooling *et al.* 2009). OMVs are potent virulence factors of pathogenic diderm bacteria. These vesicles contain toxins, DNA, immunomodulatory compounds, communication factors and adhesins, and have been associated with cytotoxicity, bacterial attachment, intercellular DNA transfer and invasion (Dorward *et al.* 1989; Kuehn & Kesty 2005; Ellis & Kuehn 2010; Maldonado *et al.* 2011; Brown *et al.* 2014). Increased vesiculation has been linked to bacterial stress and may play a role in carrying away toxic compounds, phages or unfolded proteins after exposure to stressful conditions (McBroom & Kuehn 2007; Macdonald & Kuehn 2013).

A new type of membrane vesicle was recently described for the Antarctic bacterium *Shewanella vesiculosa* M7T. These vesicles are referred to as outer-inner membrane vesicles (O-IMVs) and have a more complex double-layer structure. The protrusion of both outer and plasma membranes pulls cytoplasmic components, such as DNA and adenosine triphosphate (ATP), into the vesicles. Further studies confirmed that

O-IMVs are also secreted by diderm pathogenic bacteria such as *Neisseria gonorrhoeae, Pseudomonas aeruginosa* PAO1 and *Acinetobacter baumannii* AB41. The authors propose that these O-IMVs may be involved in lateral gene transfer and the transfer of cytoplasmic proteins (Pérez-Cruz *et al.* 2013, 2015).

Planctomycetes form a distinct phylum of the domain bacteria and possess unusual features such as intracellular compartmentalization and proteinaceous cell walls (Lee *et al.* 2009). Cells of the genus *Gemmata* also contain a membrane-bound nucleoid resembling the eukaryotic nucleus. In addition, an ability to take up proteins from the external medium through a process that is associated with internal vesicle formation was recently demonstrated for *Gemmata obscuriglobus* and is reminiscent of eukaryotic endocytosis (Lonhienne *et al.* 2010; Fuerst & Sagulenko 2011). Electron tomography of *G. obscuriglobus* cells highlighted vesicle-like structures in the periplasm. Some of these vesicle-like structures were connected to each other, forming a continuous membrane organization within the periplasm. In addition, these vesicles contain ribosomes, suggesting continuity with the cytoplasm.

More recently EMVs have been observed in bacteria lacking an OM (referred to as monoderm bacteria) from the phylum Firmicutes. EMVs produced by these bacteria are derived from the cytoplasmic membrane and are 20–250 nm in diameter (Lee *et al.* 2009; Schrempf *et al.* 2011). It has been suggested that the vesicles produced by monoderm bacteria are like their diderm counterparts, involved in pathogenesis (Rivera *et al.* 2010; Prados-Rosales *et al.* 2011). Enzymes involved in peptidoglycan degradation, antibiotic degradation, virulence factors (anthrolysin, anthrax toxin components, coagulases, hemolysins and lipases) and immunologically-active compounds have been identified in these vesicles (Marsollier *et al.* 2007; Lee *et al.* 2009; Rivera *et al.* 2010; Gurung *et al.* 2011; Prados-Rosales *et al.* 2011; Thay *et al.* 2013; Brown *et al.* 2014).

It has been shown that bacteria, produce EMVs not only in the laboratory but also in biofilms and during infections (Schooling & Beveridge 2006; Deatherage & Cookson 2012). However, until recently the presence of EMVs in natural

environments has been largely ignored. A recent study by Biller *et al.* (2014) highlighted the abundance of bacterial vesicles, from the marine phototrophic bacteria *Prochlorococcus*, in marine ecosystems as well as in the laboratory. The authors succeeded in isolating EMVs from two very different ocean samples, with concentrations ranging from 10^5 to 10^6 vesicles ml^{-1} of sea water.

These findings have important implications for microbial ecology as vesicles associated with DNA can mimic viral particles in epifluorescence microscopy (Forterre *et al.* 2013). The presence of abundant membrane vesicles besides true virions could explain the presence of bacterial sequences in environmental viromes. Moreover, since membrane vesicles can also contain viral genomes, they could even contribute to the viral component of viromes. Discriminating between virions and vesicles (some of them containing viral or plasmid DNA) appears to be a major challenge for future studies in viral ecology (Soler *et al.* 2015). In Eukarya, some virus-infected cells produce EMVs harbouring viral proteins and/or mRNA and microRNA (Pegtel *et al.* 2010; Meckes & Raab-Traub 2011). This suggests that viral vesicles (containing genomic DNA or RNA) could also exist in Eukarya.

Physiological interactions between EMVs and viruses have also been reported recently. As early as 1978, work done by Loeb *et al.* demonstrated a dramatic increase in OM production and release in the presence of T4 phage in *Escherichia coli* (Loeb & Kilner 1978). More recently, Manning and Kuehn showed that co-incubation of T4 bacteriophage and OMVs showed fast and irreversible binding. The efficiency of T4 infection was significantly reduced by the formation of complexes with the OMVs suggesting that EMVs can protect bacteria against viral infection by serving as traps for virions (Manning & Kuehn 2011). In addition, Biller *et al.* demonstrated that when purified *Prochlorococcus* vesicles were mixed with the cyanophage (PHM-2) the phage bound to vesicles. Intriguingly many vesicle-attached phage had a shortened stalk and altered capsid staining density, suggesting that they had injected their DNA into the vesicle (Biller *et al.* 2014).

EMVs in Archaea

Most archaeal species are monoderm with a cytoplasmic membrane surrounded by a crystalline protein S layer (Ellen *et al.* 2010; Albers & Meyer 2011). Only a few archaeal groups are diderm (Klingl 2014). The only diderm archaea that has been studied in detail is *Ignicoccus hospitalis* (Huber *et al.* 2002; Küper *et al.* 2010). The volume of *Ignicoccus* inter-membrane space can be large (20–1000 nm) and contains numerous vesicles that bud from the inner membrane and fuse with the OM (Näther & Rachel 2004).

Curiously, all archaea that possess a double membrane are found in close association with other organisms (Archaea, Bacteria and Eukarya) (Perras *et al.* 2014). *Ignicoccus hospitalis* usually harbours several cells of the tiny archaeon *Nanoarchaeum equitans* on its surface (Huber *et al.* 2002). *N. equitans* has the smallest known genome size for an archaeon (0.49 Mb) and cannot synthesize many essential components,

including lipids (Waters *et al.* 2003). It is assumed that these components could be delivered from the cytoplasm of *I. hospitalis* to *N. equitans* via vesicles that reach the OM at the level of the symbiont attachment.

Monoderm archaea also produce EMVs. The earliest reports for archaeal EMVs came from the studies carried out on crenarcheal *Sulfolobus islandicus* (Prangishvili *et al.* 2000). These vesicles, 90–230 nm in diameter and coated with an S layer, were shown to be associated with an antimicrobial protein, termed 'sulfolobicin', that inhibits the growth of related *Sulfolobus* species. Proteomic analyses showed that the lipid and protein profiles of parent cells and secreted vesicles were different (Ellen *et al.* 2009).

A study carried out to screen for novel viruses among the euryarchaeal order of Thermococcales revealed that most of the strains tested released small spherical vesicles (Soler *et al.* 2008; Gaudin *et al.* 2013; Marguet *et al.* 2013). These vesicles (50–150 nm) are produced by a protruding of the cell envelope along with the S layer and frequently form rows of EMVs ressembling nanopods or nanotubes observed in Bacteria and sometimes can connect cells together (Marguet *et al.* 2013).

A closer look at the protein profiles of EMVs from two species of Thermococcales, *Thermococcus gammatolerans* and *Thermococcus kodakaraensis* showed that both EMVs and cell membranes from the same species have a similar composition. This is in contrast to the results obtained by Ellen *et al.* (2009) for *Sulfolobus*. The major protein present in cell membranes and EMVs of both species is the oligopeptide binding protein OppA (Gaudin *et al.* 2013) which is also found in *Sulfolobus* EMVs (Ellen *et al.* 2009). EMVs produced by Thermococcales often have genomic DNA associated with them (Soler *et al.* 2008). It appears that the DNA is afforded a degree of protection by the vesicles and is more resistant to thermodenaturation. This could be vital for horizontal gene transfer in hyperthermophilic archaea, which thrive in temperatures that are not so kind to DNA. The group went on to demonstrate that EMVs transfer DNA between cells using the genetically tractable strain *T. kodakaraensis* KUW1 (Gaudin *et al.* 2013). Interestingly, EMVs produced by *Thermococcus nautili* harbour the genome of the plasmid pTN1 and of a defective virus named pTN3 suggesting a possible role in gene transfer between species and generation of new viral forms (Soler *et al.* 2011; Gaudin *et al.* 2014).

Viruses and EMVs

Interestingly, the morphology of EMVs is strongly reminiscent of the morphology of some virions. It is thus tempting to make a link between the origin of the first protocell via the formation of vesicles and the origin of viruses. The origin of viruses is a fascinating and important topic as metagenomic analyses have shown that viral genomes represent the major source of genetic information in the biosphere (Suttle 2005; Rohwer & Thurber 2009; Kristensen *et al.* 2010). Viruses are extremely diverse and each one of the three domains of life is associated with a specific ensemble of viral lineages (Pina *et al.* 2011; Forterre *et al.* 2014). Viruses are also very ancient, predating the last

universal common ancestor (LUCA), since some major viral lineage defined by homologous capsid proteins and packaging ATPases are present in these three ensembles (Abrescia *et al.* 2012). Three types of hypotheses have been put forward to explain this origin: the '*virus first*' hypothesis in which viruses originated before cells, the '*regression hypothesis*', in which cells or proto-cells evolved into virions by regressive evolution and the '*escape hypothesis*', in which fragments of cellular genomes (either from prokaryotes or eukaryotes) became infectious (Forterre 2006, 2010). Forterre and Krupovic recently re-evaluated the escape hypothesis (Forterre & Krupovic 2012). The updated version of this hypothesis proposes that viruses originated by transformation of ancient ribocells (cell encoding ribosome and dividing by binary fission) into a ribovirocell, still dividing but producing virions carrying selfish RNA replicons are capable of infecting other ribocells, and later on into virocells (cell producing virions) killing their host ribocells. In the classical version of the escape hypothesis, these ribocells are confused with modern cells (prokaryotes or eukaryotes). In the updated version of the escape hypothesis, these ribocells were ancestral RNA-based cells that antedated LUCA (refer Fig. 1). Furthermore, in the classical version of the escape hypothesis, the focus was on the viral genome, with the origin of virions put aside; the 'modern escape hypothesis' focuses on the virion (Forterre & Krupovic 2012). The origin of viruses should not be confused with the origin of viral genomes *per se*, the latter being in fact the history of replicons. To understand the origin of viruses, one should focus on the origin of the mechanisms of virion production by virocells (how they are formed, excreted from the cell and how they can transfer their genomic information into cells). Viral genomes may have originated from ancestral selfish replicons present in ancient ribocells and virions from micro-compartments, nucleoprotein complexes or EMVs present in ancient ribocells (Forterre & Krupovic 2012).

The production of EMVs by ancient cells or proto-cells could have thus played a role in the origin of some of the very first viral lineages before LUCA (Jalasvuori & Bamford 2008). Intriguingly, vesicles have many characteristics in common with enveloped viruses including biophysical properties, formation and uptake by cells. It is clear that some viruses utilize vesicle secretion pathways during infection. For example, retroviruses recruit several elements from the vesicle biogenesis pathways for functional virus release suggestive of a possible viral origin of the microvesicle system or perhaps of an evolutionary conserved system of virus-vesicle co-dependence (Izquierdo-Useros *et al.* 2011). Are EMVs predecessors of viruses or do the viruses merely exploit the same cellular machinery? (Gould *et al.* 2003; Pelchen-Matthews *et al.* 2004). Analysis of EV cargo revealed that many of the molecules found have been implicated in virus binding and entry (György *et al.* 2011). The diversity of EMV populations and components suggests that EMVs enter cells through various mechanisms similar to the multiple pathways identified for viruses (Marsh & Helenius 2006). Understanding the interplay between viruses and EMVs could shed some light on the origin of EMVs and or origin of viruses. Our current mechanistic understanding of EMV biology and function, especially in regard to virus infection, is in its infancy. However, the obviously broad biological, medical and evolutionary implications of EMVs make them a significant and exciting area of research.

LUCA, the origin of life and EMVs

The fact that all cells tested from the three domains of life produce EMVs suggests that this is an ancient process that possibly dates back to the LUCA, or beyond. LUCA is the most recent organism from which all modern cells derive. LUCA should not be confused with the first cell, but was the product of a long period of evolution. Being the 'last' means that LUCA was preceded by a long succession of older 'ancestors.' A plethora of cellular lineages that have left no descendants today may have existed before LUCA. It is important to consider that many of these were probably still present at the time of LUCA, and some have probably even coexisted for some time with its descendants, possibly contributing via horizontal gene transfer to some traits present in modern lineages (Forterre & Gribaldo 2007).

Due to advances in comparative genomics we can infer some of the characteristics of LUCA by comparing what present-day organisms have in common. The key assumption is that genes shared by many diverse extant species are most likely to be inherited from their common ancestor. The 'core' set of genes is very small and nearly all of them encode proteins involved in translation and the core transcription machinery. If the genes for rRNAs and tRNAs are included, the universal set comprises about 100 genes (Harris *et al.* 2003; Koonin 2003; Charlebois & Doolittle 2004; Delaye *et al.* 2005). Beside proteins involved in translation and transcription, this small gene set includes a few critical proteins involved in the makeup of membranes, such as membrane bound ATPases, signal recognition particles involved in the translation of membrane proteins and proteins involved in protein secretion. It is thus now clear that LUCA was a cellular organism that already harboured a sophisticated translation apparatus and had a cytoplasmic membrane (Pereto *et al.* 2004; Jekely 2006; Forterre & Gribaldo 2007).

In living cells today, cell membranes perform varied roles despite the fact that they are primarily considered necessary to partition cells. These roles include: energy transduction, nutrient and ion transport, signal transduction and certain metabolic reactions. The latter include the synthesis of the membrane lipids themselves to allow growth of the bilayers in different cellular compartments (Segré *et al.* 2001). On the early Earth, they also provided 'micro-environments' enabling the confinement of molecular information and organic resources essential for the beginning of cellular life. This confinement helped to prevent leakage, concentrate substrates to promote reactions and isolate successful RNA replicators from unsuccessful ones (Koch 1985; Deamer 1997; Segré *et al.* 2001). The RNA world would be difficult to achieve from simple organic molecules dissolved in the primordial soup (Joyce 1991; Deamer *et al.* 2002). Random concentration of the molecules may have occurred due to processes such as evaporation, eutectic freezing, or adsorption to mineral
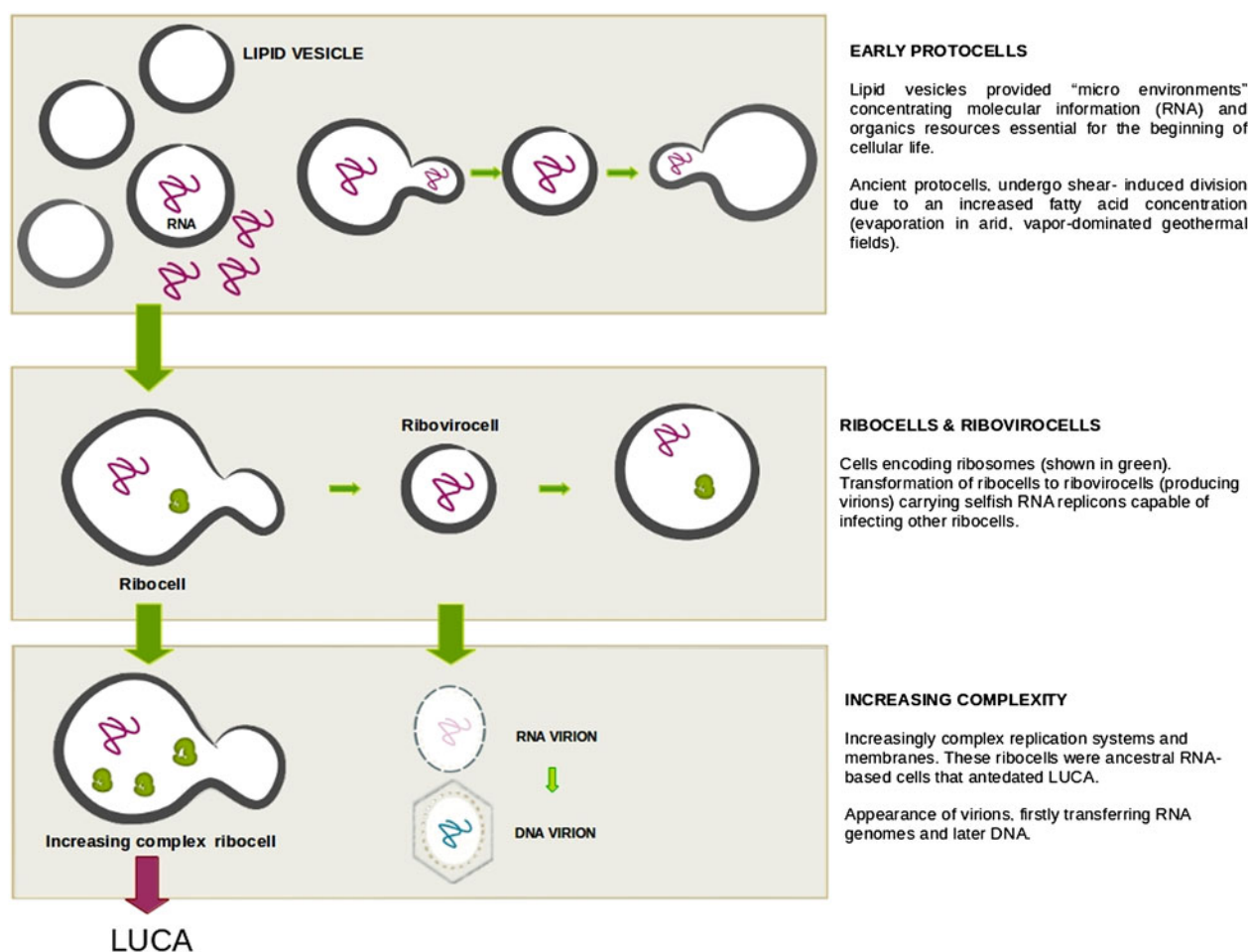
Fig. 1. Role of membrane vesicles in early cellular evolution and how they would have helped shape the nature of LUCA.

surfaces (Wächtershäuser 1988; Stribling & Miller 1991; Robertson & Miller 1995; Ferris *et al.* 1996; Kanavarioti *et al.* 2001; Sowerby & Petersen 2002). However, these processes on their own would be thermodynamically inefficient to account for the origin of life beyond the initial stages (Deamer *et al.* 2002).

Since compartmentalization is a key issue in the early evolution of life many researchers have tried to reconstruct the habitats of the earliest life forms where, under natural conditions, small cell-like compartments could be formed in large numbers over an extended period of time. Mulkidjanian *et al.* combined geochemical evidence with ionic composition of the modern cells, with a particular emphasis on their universal preference for K + ions over Na + ions. They suggest that the first cells could have emerged at inland geothermal fields within ponds of condensed and cooled geothermal vapour (Mulkidjanian *et al.* 2012).

Schreiber *et al.* presented a model for the origin of life in tectonic fault systems in the continental crust (Schreiber *et al.* 2012). These vast networks (from sub-mm to several meters) of interconnected cracks and cavities are mainly filled with water and carbon dioxide. They also provide a wide variety of hydrothermal chemistry and numerous catalytic surfaces

and thus may offer ideal reaction conditions for prebiotic chemistry (Schreiber *et al.* 2012). The authors also predicted that vesicle formation is expected to occur in these tectonic fault zones at a depth where pressure and temperature conditions induce a phase transition between supercritical scCO2 and subcritical gaseous CO_2 (gCO_2). The group went on to attempt reproducing experimentally vesicle formation with a lipid in a water/CO_2 system (Mayer *et al.* 2015).

Lipidic vesicle models require specific conditions allowing the vesicles to emerge and persist long enough, including a continuous flow of matter and energy and the possibility for the waste products to be diluted in an open milieu so that the system is not hindered by their increasing concentration (Tessera 2009, 2011). The serpentinite-hosted Lost City Hydrothermal Field (Kelley *et al.* 2005) is a place that has all these conditions. At high temperatures, lipid compounds can be produced by aqueous Fischer–Tropsch-type synthesis (Rushdi & Simoneit 2001). Such abiogenic production of short-chain hydrocarbons was found at this site (Proskurowski *et al.* 2008). Vesicles with membranes composed of bi-layers from mixtures of amphiphilic and hydrophobic molecules could have formed from the organic compounds present locally at high concentrations. However, the stability of bilayer lipidic membranes at high

pressure and temperature is questionable as bilayers formed of simple amphiphiles are extremely fragile at high pH, ionic strength and high temperatures (Deamer *et al.* 2002; Maurer *et al.* 2009; Tessera 2011).

Recent work by a team of researchers at the University of Strasbourg (France) provides a credible scenario for the origin of life. They showed that the dynamics of synthetic reactions could be enhanced by compartmentalization in micrometre-sized droplets. The enhancement occurs due to the fact that the reactants are adsorbed to the droplet surface, react and diffuse back into the interior of the droplet. The surface tension inside the droplets drives the formation of bonds between the reactants. As a model system, they used the reversible reaction of a non-fluorescent amine; (1) with a very weakly fluorescent aldehyde, (2) to form a fluorescent imine and (3) in water (Fallah-Araghi *et al.* 2014). The choice of non-fluorescent reactants and fluorescent product allowed for easy visualization of the chemical reaction using fluorescence imaging. Indeed, the compartmentalization of the primordial soup into vesicles is thought to have favoured chemical synthesis that resulted in the emergence of primitive life forms.

Membranes can self-assemble into vesicular structures when small amphiphilic molecules spontaneously associate by hydrophobic interactions. Studies have demonstrated that fatty acids spontaneously assemble into bilayer membranes, building vesicles able to grow by incorporation of free lipid molecules and divide (Dworkin *et al.* 2001; Deamer *et al.* 2002; Hanczyc *et al.* 2003). Simple fatty acid vesicles, studied as models of ancient protocells, undergo shear- induced division when their surface to volume ratio is artificially increased by addition of fatty acids (Zhu & Szostak 2009). Bacterial mutants with increased lipid synthesis and excess membrane production also show a physical mode of division (Mercier *et al.* 2013). Under primordial conditions, this increased fatty acid concentration may have been achieved by evaporation in arid, vapour-dominated geothermal fields that have been identified as plausible hatcheries for the emergence of cells (Mulkidjanian *et al.* 2012). Mineral surfaces, such as montmorillonite, also stimulate the formation of lipid vesicles (Hanczyc *et al.* 2007). Vesicles encapsulating RNA have been shown to grow preferentially by lipid capture at the expense of empty vesicles (Chen *et al.* 2004; Chen & Szostak 2004). The osmotic pressure is higher inside RNA-containing vesicles due to the counter ions screening the negative charges of RNA. This osmotic pressure is counterbalanced by membrane tension, driving the uptake of fatty acids. At an early stage, this mechanism could have favoured vesicles containing charged molecules, such as ribose phosphate and/or polyphosphate, over those containing neutral molecules. Later on, the encapsulation of RNA replicators would have induced a primitive form of competition between the first RNA cells, since those containing more efficient replicators would have grown faster (Chen *et al.* 2004; Forterre & Gribaldo 2007). Finally, vesicle membrane growth generates a trans-membrane pH gradient (Chen & Szostak 2004; Forterre & Gribaldo 2007), suggesting that some universal features of the living world could have their origin in fundamental physicochemical features.

Conclusions

EMVs have been implicated in many aspects of cellular life in all three domains. In particular the role of EMVs in inter-cellular communication is currently receiving much attention. Virus-infected cells proved useful in early studies to elucidate the role of EMV release in inter-cellular communication. The convergence of EMV biogenesis and certain aspects of viral assembly and release pathways suggests a possible viral origin of the EMV system or perhaps of an evolutionary conserved system of virus-vesicle co-dependence.

The fact that release of EMVs is a process conserved in all three domains of life implies an important evolutionary link, one that possibly dates back to the LUCA. It is widely believed that the cytoplasm evolved inside a primordial lipid vesicle. On early Earth the key components of the primitive cell were a genome, a metabolic system and the cell membrane. Indeed, a membrane-protected micro-environment would have been a key to the survival of spontaneous molecular systems and efficient metabolic systems. Efforts to synthesize model protocells encapsulating self-replicating molecules and metabolic systems may one day enable us to test these hypotheses and gain a better understanding into the origin of life.

Acknowledgements

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